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Notes on Methodology

A modified preparative ultracentrifuge technique for separating human serum lipoproteins*

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» Preparative ultracentrifugation of human serum to separate lipoproteins into recoverable fractions which are amenable to quantitative analysis of their constituents is a valuable laboratory tool. It is desirable for laboratories using direct methods of measuring cholesterol and phospholipids to be able to quantify without having to rely on time-consuming dialysis or extraction. It is the purpose of this paper to show that sodium sulfate, rather than sodium chloride or a sodium chloride-potassium bromide mixture, should be used to adjust solvent density if a direct procedure for measuring cholesterol is to be used.

The preparative ultracentrifugal procedure was comparable to that of Havel *et al.* (1). The Spinco Model L was used with a 40.3 rotor at 38,000 rpm for 16 hours at 2°. Two 2-ml serum samples for each subject were centrifuged simultaneously, one at a solvent density of 1.006 g/ml, the other at 1.063 g/ml.

Aqueous Na_2SO_4 solutions for controlling solvent density:

Density 20°	Concentration, g/liter	Molarity
1.006	8.6	0.061
1.063	76.73	0.54
1.12	144.85	1.02

The 1.006 g/ml sample was prepared by measuring the serum into a lusteroid tube and then filling with 1.006 g/ml salt solution. The 1.063 g/ml sample was prepared by diluting the serum with an equal volume of 1.12 g/ml salt solution and then filling the tube with the salt solution of 1.063 g/ml. The Spinco Tube Slicer was used to recover the fractions.

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Chemical analyses were performed on three of the four fractions obtained, namely: $d > 1.063$, $d < 1.063$, and $d < 1.006$. The cholesterol procedure used was that described by Zlatkis *et al.* (2) with the color reagent modification described by Rosenthal *et al.* (3). The phospholipid procedure used was that described by Zilversmit and Davis (4). Reproducibility and recovery of cholesterol and phospholipids from the ultracentrifugally separated lipoproteins using sodium sulfate were comparable to those reported by Havel *et al.* (1).

To determine the effects of added salts on the direct determination of cholesterol, equal portions of standard, of serum, and of the lipoprotein fractions were diluted with an equal volume of either NaCl or Na_2SO_4 solutions. The lipoprotein fractions were separated using Na_2SO_4. The concentrations of the added salt solutions are shown in the abscissas in Figure 1. They are expressed in density units so that comparison with solvent densities used in ultracentrifugation may be made. Sodium chloride caused very significant errors, whereas sodium sulfate had virtually no effect on cholesterol values. Neither salt affected phospholipid determinations.

It is not understood why increasing the sodium chloride affects the cholesterol determination. One hypothesis is that the excess sodium chloride reacts with the ferric chloride to increase the concentration of a compound with the formula Na_2FeCl_5 (5). When separated from aqueous solution, other comparable alkaline metallic chlorides crystallize with $FeCl_3$ to give compounds with the general formula $M_2FeCl_5 \cdot H_2O$. It may be that under the anhydrous conditions of the test, cholesterol takes the place of the water and that basically the compound measured is $Na_2FeCl_5 \cdot$cholesterol. As the chloride concentration increases, the formation of this cholesterol compound would rise to

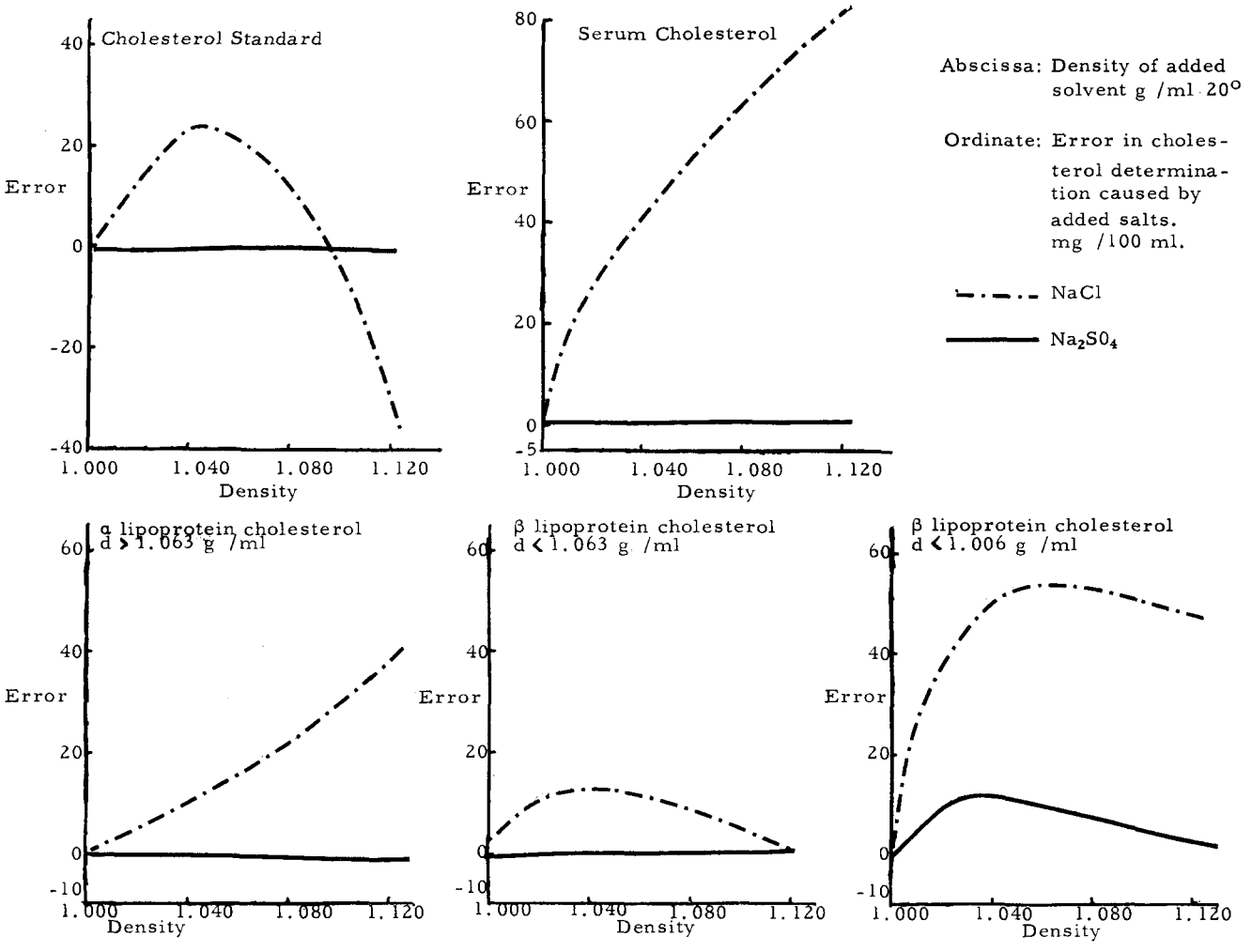

FIG. 1. Error in cholesterol determinations caused by added salts.

a maximum, then decrease as the formation of the complex compound would be inhibited by the excess chloride competing to form Na_3FeCl_6 or some higher complex. In Figure 1 the curve given by the standard solution would be explained by this hypothesis.

The whole serum and the d > 1.063 lipoprotein cholesterol curves do not follow this hypothesis unless the following reasoning is used. They both have a relatively high concentration of protein which must successfully compete for the excess sodium chloride, thus preventing the formation of a maximum color in the chloride range given. The fact that protein concentrations in the serum lipoprotein fractions d < 1.063 and d < 1.006 are relatively small and that maxima are reached, tends to confirm this reasoning.

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